

WE CLAIM:

1. An electrochemical sensor, comprising:  
a substrate;  
a recessed channel formed in a surface of the substrate; and  
a conductive material disposed in the recessed channel and forming a working electrode.
2. The electrochemical sensor of claim 1, further comprising an electron transfer agent disposed on the working electrode to transfer electrons between the working electrode and the analyte.
3. The electrochemical sensor of claim 2, further comprising a catalyst wherein the electron transfer agent transfers electrons between the working electrode and the analyte via the catalyst.
24. The electrochemical sensor of claim 2, wherein the electron transfer agent is nonleachably disposed on the working electrode.
25. The electrochemical sensor of claim 2, wherein the electron transfer agent is immobilized on the working electrode.
26. The electrochemical sensor of claim 2, wherein at least 90% of the electron transfer agent remains disposed on the working electrode after immersion in interstitial fluid at 37°C for 24 hours.
27. The electrochemical sensor of claim 6, wherein at least 99% of the electron transfer agent remains disposed on the working electrode after immersion in interstitial fluid at 37°C for 24 hours.

*28.* 8. The electrochemical sensor of claim 6, wherein at least 90% of the electron transfer agent remains disposed on the working electrode after immersion in interstitial fluid at 37°C for 72 hours.

*29.* 9. The electrochemical sensor of claim 2, wherein the electron transfer agent is immobilized in a sol-gel material.

*10.* 10. The electrochemical sensor of claim 1, wherein the electrochemical sensor is configured for *in vivo* operation.

*11.* 11. The electrochemical sensor of claim 1, wherein the electrochemical sensor is configured for *in vitro* operation.

*12.* 12. The electrochemical sensor of claim 1, wherein the substrate is a polymeric material.

*13.* 13. The electrochemical sensor of claim 1, wherein the substrate is flexible.

*14.* 14. The electrochemical sensor of claim 1, wherein the substrate is planar.

*15.* 15. The electrochemical sensor of claim 1, wherein the surface of the substrate comprises a wide portion and a narrow portion, the narrow portion being configured for implantation into a patient.

*30.* 16. The electrochemical sensor of claim 15, wherein the narrow portion has a width of 0.5 mm or less.

*3.* 17. The electrochemical sensor of claim 1, wherein the recessed channel has a width of 250 µm or less.

*4.* 18. The electrochemical sensor of claim *X*, wherein the recessed channel has a width of 100 μm or less.

*5.* 19. The electrochemical sensor of claim *X*, wherein the recessed channel has a width of 50 μm or less.

*6.* 20. The electrochemical sensor of claim 1, wherein the conductive material comprises a metal.

*7.* 21. The electrochemical sensor of claim 1, wherein the conductive material comprises carbon.

*8.* 22. The electrochemical sensor of claim 1, further comprising a catalyst for catalyzing a reaction of an analyte, the catalyst being disposed proximate to the working electrode.

*9.* 23. The electrochemical sensor of claim *22*, wherein the catalyst is disposed on the working electrode.

*10.* 24. The electrochemical sensor of claim *22*, further comprising an electron transfer agent disposed on the working electrode.

*11.* 25. The electrochemical sensor of claim *24*, wherein at least a portion of the catalyst is in contact with the electron transfer agent.

*12.* 26. The electrochemical sensor of claim *22*, wherein the catalyst is nonleachably disposed within the electrochemical sensor.

11. 27. The electrochemical sensor of claim 22, wherein the catalyst is immobilized within the electrochemical sensor.
12. 28. The electrochemical sensor of claim 22, wherein the catalyst comprises an enzyme.
13. 29. The electrochemical sensor of claim 28, wherein the enzyme is an oxidase or a dehydrogenase.
14. 30. The electrochemical sensor of claim 29, wherein the analyte is glucose and the enzyme is oligosaccharide dehydrogenase, PQQ-glucose dehydrogenase, or glucose oxidase.
15. 31. The electrochemical sensor of claim 22, further comprising a second catalyst for catalyzing a reaction of a product compound formed in the reaction of the analyte.
16. 32. The electrochemical sensor of claim 31, wherein the product compound comprises hydrogen peroxide and the second catalyst comprises a peroxidase.
33. The electrochemical sensor of claim 1, further comprising a biocompatible coating disposed over at least a portion of the working electrode.
34. The electrochemical sensor of claim 1, further comprising a mass transport limiting layer disposed over at least a portion of the working electrode to limit transport of an analyte to the working electrode.
35. An analyte responsive electrochemical sensor comprising a working electrode and a mass transport limiting membrane, which mass transport limiting membrane maintains a rate of permeation of the analyte through the mass transport

limiting membrane with a variation of no more than 3% per °C at temperatures ranging from 30°C to 40°C.

36. The electrochemical sensor of claim 34, wherein a rate of permeation of the analyte through the mass transport limiting layer varies by no more than 1% per °C for temperatures ranging from 30°C to 40°C.

37. The electrochemical sensor of claim 34, wherein the mass transport limiting layer comprises a membrane having a plurality of track etched pores.

38. The electrochemical sensor of claim 34, wherein the mass transport limiting layer absorbs 5 wt.% or less of water at 37°C when in contact with interstitial fluid for 24 hours.

39. The electrochemical sensor of claim 4, further comprising a second recessed channel and a second conductive material disposed in the recessed channel, the second conductive material forming a counter electrode.

40. The electrochemical sensor of claim 39, wherein the second conductive material is the same as the first conductive material.

41. The electrochemical sensor of claim 39, wherein the counter electrode is also a reference electrode.

42. The electrochemical sensor of claim 4, further comprising a third recessed channel and a third conductive material disposed in the channel, the third conductive material forming a reference electrode.

43. The electrochemical sensor of claim 42, wherein the third conductive material is the same as the first conductive material.

*11.*  
44. The electrochemical sensor of claim 42, wherein the third conductive material comprises a silver species.

*18.*  
45. The electrochemical sensor of claim 1, further comprising an additional recessed channel with conductive material disposed in the additional recessed channel to form an additional working electrode.

*13.*  
46. The electrochemical sensor of claim 45, wherein a sensing layer is disposed proximate to the additional working electrode.

*14.*  
47. The electrochemical sensor of claim 45, wherein the additional working electrode is configured within the electrochemical sensor to provide a background signal.

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48. The electrochemical sensor of claim 1, further comprising a temperature probe disposed on the substrate, the temperature probe having a plurality of spaced-apart probe leads and a temperature-dependent element in contact with the spaced-apart probe leads, the temperature-dependent element comprising a material having a temperature-dependent characteristic that produces a change in a signal of the temperature probe in response to a change in temperature.

*42.*  
49. The electrochemical sensor of claim 48, wherein the temperature-dependent characteristic comprises a resistance of the probe element.

*43.*  
50. The electrochemical sensor of claim 48, wherein the material of the temperature-dependent element comprises carbon.

*44.*  
51. The electrochemical sensor of claim 48, wherein the temperature-dependent element comprises a conductive material with a smaller cross-section than the probe leads.

*45.*  
52. The electrochemical sensor of claim 48, wherein the temperature-dependent element is disposed in a recessed channel on a surface of the substrate.

*46.*  
53. The electrochemical sensor of claim 48, wherein the probe leads are disposed in recessed channels on a surface of the substrate.

54. The electrochemical sensor of claim 1, wherein the analyte is glucose.

55. The electrochemical sensor of claim 1, wherein the electrochemical sensor is configured for implantation into an animal and an anticoagulant agent is disposed on a portion of the substrate that is configured for implantation.

56. The electrochemical sensor of claim 1, wherein the substrate is flexible.

57. The electrochemical sensor of claim 1, further comprising laccase disposed proximate to the working electrode to monitor a level of oxygen in a fluid.

*Sed 1*  
58. An electrochemical sensor, comprising:  
a substrate;  
a plurality of recessed channels formed in at least one surface of the substrate;  
and

a conductive material disposed in each of the recessed channels, the conductive material in at least one of the recessed channels forming a working electrode.

59. The electrochemical sensor of claim 58, wherein the substrate is flexible.

60. The electrochemical sensor of claim 58, wherein a width of the recessed channel forming the working electrode is 250 $\mu$ m or less.

61. The electrochemical sensor of claim 58, wherein a width of the recessed channel forming the working electrode is 100 $\mu$ m or less.

62. The electrochemical sensor of claim 58, wherein a width of the recessed channel forming the working electrode is 50 $\mu$ m or less.

63. The electrochemical sensor of claim 58, further comprising an electron transfer agent disposed on the working electrode.

64. The electrochemical sensor of claim 58, further comprising a catalyst disposed proximately to the working electrode to catalyze a reaction of the analyte.

65. The electrochemical sensor of claim 58, wherein the plurality of recessed channels are formed in a same surface of the substrate.

66. The electrochemical sensor of claim 58, wherein at least two of the plurality of recessed channels are formed on different surfaces of the substrate.

67. The electrochemical sensor of claim 66, wherein the different surfaces of the substrate are opposing surfaces of the substrate.

68. The electrochemical sensor of claim 58, further comprising a counter electrode formed in one of the plurality of recessed channels.

69. The electrochemical sensor of claim 58, further comprising a reference electrode formed in one of the plurality of recessed channels.

*Sab 1*

70. The electrochemical sensor of claim 69, wherein the reference electrode is formed on a first surface of the substrate and the working electrode is formed on a second surface of the substrate, the first and second surfaces being opposing surfaces of the substrate.

*83,* *84*  
71. The electrochemical sensor of claim 70, further comprising a via formed in the substrate between the first and second surfaces, conductive material being disposed in the via and in conductive communication with a one of the working electrode or reference electrode.

*21.* *72.* The electrochemical sensor of claim 58, further comprising a temperature probe disposed on a surface of the substrate.

*22.* *73.* The electrochemical sensor of claim 72, wherein the temperature probe is disposed on a first surface of the substrate and the working electrode is disposed on a second surface of the substrate.

74. A method of determining a level of an analyte in a fluid, the method comprising:

contacting the fluid with an electrochemical sensor, the sensor comprising a substrate, a recessed channel formed in the substrate, and conductive material deposited in the recessed channel to form a working electrode;

generating an electrical signal in the sensor in response to the presence of the analyte; and

determining a level of the analyte from the electrical signal.

*51.* *75.* The method of claim 74, wherein contacting the fluid with an electrochemical sensor comprises implanting the electrochemical sensor in an animal so that the electrochemical sensor is in contact with a body fluid of the animal.

*52.*

76. The method of claim 25, wherein implanting the electrochemical sensor in an animal comprises subcutaneously implanting the electrochemical sensor in the animal so that the electrochemical sensor is in contact with the interstitial fluid of the animal.

*51.*

*53.*

77. The method of claim 74, wherein contacting the fluid with an electrochemical sensor comprises contacting an *in vitro* sample of a body fluid with the electrochemical sensor.

*50.*

78. The method of claim 21, wherein the *in vitro* sample has a volume of less than 1  $\mu\text{L}$ .

*53.*

79. A temperature sensor, comprising:  
a substrate;  
a recessed channel formed on the surface of the substrate; and  
a temperature probe disposed in the recessed channel, the temperature probe comprising two probe leads disposed in spaced apart portions of the recessed channel and temperature-dependent element disposed in the recessed channel and in contact with the two probe leads, the temperature-dependent element comprising a material having a temperature-dependent characteristic that changes a signal of the temperature probe in response to a change in temperature.

*80.*

80. The temperature sensor of claim 79, wherein the temperature-dependent characteristic is a resistance of the probe element.

*81.*

81. An electrochemical sensor for determining a level of an analyte in a fluid, comprising:  
a substrate;

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a recessed channel formed in a surface of the substrate;  
a conductive material disposed in the recessed channel and forming a working electrode; and  
a catalyst disposed proximally to the working electrode to catalyze a reaction of the analyte resulting in a change in a level of a second compound;  
wherein the electrochemical sensor is responsive to the level of the second compound in the fluid.

*85*  
*81*  
82. The electrochemical sensor of claim *81*, further comprising a second catalyst which catalyzes a reaction of the second compound to generate a signal at the working electrode.

*86*  
*81*  
83. The electrochemical sensor of claim *81*, wherein the second compound comprises a co-reactant in the reaction of the analyte.

*81*  
*81*  
84. The electrochemical sensor of claim *81*, wherein the second compound comprises a product compound of the reaction of the analyte.

85. A method of determining a level of an analyte in a fluid, comprising:  
contacting the fluid with an electrochemical sensor, the electrochemical sensor having a substrate, a recessed channel formed in a surface of the substrate, a conductive material disposed in the recessed channel and forming a working electrode, and a catalyst disposed proximally to the working electrode;

changing a level of a second compound in the fluid by a reaction of the analyte catalyzed by the catalyst;

generating a signal in response to the level of the second compound; and  
determining the level of the analyte from the signal.

86. The method of claim 85, wherein the second compound is a co-reactant with the analyte and changing the level of the second compound comprises decreasing the level of the second compound by reaction of the second compound with the analyte.

87. The method of claim 86, wherein the analyte is glucose and the second compound is oxygen.

88. The method of claim 85, wherein the second compound is a product compound of the reaction of the analyte and changing the level of the second compound comprises increasing the level of the second compound by reaction of the analyte to form the second compound.

89. The method of claim 88, wherein the analyte is glucose and the second compound is hydrogen peroxide.

90. The method of claim 85, wherein the electrochemical sensor further comprises a second catalyst disposed proximate to the working electrode and generating a signal comprises reacting the product compound in the presence of the second catalyst.

91. The method of claim 90, wherein the second compound comprises hydrogen peroxide and the second catalyst comprises a peroxidase.

5. 92. An electrochemical sensor, comprising:

a substrate; and

a working electrode disposed on the substrate, the working electrode comprising a carbon material and having a width along at least a portion of the working electrode of 150  $\mu\text{m}$  or less.

18. <sup>51</sup> 93. The electrochemical sensor of claim 92, wherein a width along at least a portion of the working electrode is 75  $\mu\text{m}$  or less.

19. <sup>51</sup> 94. The electrochemical sensor of claim 92, wherein a width along at least a portion of the working electrode is 25  $\mu\text{m}$  or less.

20. <sup>51</sup> 95. The electrochemical sensor of claim 92, wherein the substrate is flexible.

21. <sup>51</sup> 96. The electrochemical sensor of claim 92, further comprising a second electrode disposed next to the working electrode, the second electrode and working electrode being disposed 150  $\mu\text{m}$  or less apart.

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97. The electrochemical sensor of claim 92, wherein the working electrode is disposed in a channel formed on a surface of the substrate.

22. <sup>51</sup> 98. The electrochemical sensor of claim 92, wherein the working electrode is formed by transferring, to the substrate, carbon material electrically attracted to a drum in an image of the working electrode.

23. <sup>51</sup> 99. The electrochemical sensor of claim 92, wherein the working electrode is formed by transferring, to the substrate, carbon material magnetically attracted to a drum in an image of the working electrode.

24. <sup>51</sup> 100. The electrochemical sensor of claim 92, wherein the working electrode is formed by transferring the carbon material from a film onto the substrate using a print head.

25. <sup>51</sup> 101. The electrochemical sensor of claim 92, wherein the working electrode is formed by ejecting carbon material onto the substrate.

*27.* 102. The electrochemical sensor of claim 92, wherein the working electrode is formed by depositing a curable coating on the substrate, curing portions of the curable coating to form a channel, and depositing carbon material in the channel to form the working electrode.

*103.* A subcutaneously implantable sensor, comprising:

a substrate; and

conductive material non-leachably disposed on the substrate to form a working electrode, the conductive material comprising carbon; and

an enzyme non-leachably disposed proximate to the working electrode.

*104.* An electrochemical sensor, comprising:

a substrate; and

conductive material disposed on a surface of the substrate to form a plurality of traces, at least one of the traces forming a working electrode;

wherein the plurality of conductive traces are separated on the surface of the substrate by 150  $\mu\text{m}$  or less.

*105.*

The electrochemical sensor of claim 104, wherein the plurality of conductive traces are separated on the surface of the substrate by 50  $\mu\text{m}$  or less.

*106.*

The electrochemical sensor of claim 104, further comprising conductive material disposed on a second surface of the sensor to form one or more additional conductive traces.

*107.*

An electrochemical sensor, comprising:  
a substrate; and  
conductive material disposed on a surface of the substrate to form a plurality of traces, at least one of the traces forming a working electrode;

wherein the plurality of conductive traces are disposed on the surface of the substrate at a density, along a width of the substrate, of 667  $\mu\text{m}/\text{trace}$  or less.

*98.*

~~108.~~ The electrochemical sensor of claim ~~107~~, wherein the plurality of conductive traces are disposed on the surface of the substrate at a density, along a width of the substrate, of 167  $\mu\text{m}/\text{trace}$  or less.

*91.*

~~108.~~ The electrochemical sensor of claim ~~107~~, wherein the plurality of

conductive traces are disposed on the surface of the substrate at a density, along a width of the substrate, of 167  $\mu\text{m}/\text{trace}$  or less.

*93.*

~~109.~~ An electrochemical sensor comprising:

a substrate,

conductive material disposed on the substrate to form a working electrode; and  
a contact pad disposed on the substrate and connected to the working electrode,

the contact pad comprising a non-metallic conductive material.

*94.*

~~110.~~ The electrochemical sensor of claim ~~109~~, wherein the non-metallic conductive material of the contact pad comprises carbon.

*93.*

~~111.~~ The electrochemical sensor of claim ~~109~~, wherein the working electrode is disposed on a first surface of the substrate and the contact pad is disposed on a second surface of the substrate, the substrate having a via with conductive material disposed therein, wherein the working electrode and contact pad are coupled in conductive communication through the via.

*95.*

~~112.~~ An analyte monitoring system, comprising:

a sensor having a substrate, a working electrode disposed on the substrate, and a contact pad coupled to the working electrode; and

a control unit having a conductive contact coupled to the working electrode, the control unit being configured to apply a potential across the working electrode;

wherein at least one of the contact pad and the conductive contact comprises a non-metallic material.

*97.*

~~113.~~ The analyte monitoring system of claim ~~112~~, wherein the non-metallic material comprises carbon.

*98.*

~~114.~~ The analyte monitoring system of claim ~~112~~, wherein the non-metallic material comprises graphite or vitreous carbon.

*99.*

~~115.~~ The analyte monitoring system of claim ~~112~~, wherein an other of the contact pad and the conductive contact comprises gold, palladium, a platinum group metal or ruthenium dioxide.

*100.*

~~116.~~ The analyte monitoring system of claim ~~112~~, wherein, when the contact pad and conductive contact are placed in contact, immersed in a 1 mM NaCl solution, and a potential is applied, a signal resulting from the corrosion of the contact pad and the conductive contact is 3% or less of a signal arising from electrolysis of an analyte in a solution having a concentration of the analyte within an expected physiological range.

*101.*

~~117.~~ The analyte monitoring system of claim ~~112~~, wherein, when the contact pad and conductive contact are placed in contact, immersed in a 100 mM NaCl solution, and a potential is applied, a signal resulting from the corrosion of the contact pad and the conductive contact is 3% or less of a signal arising from electrolysis of an analyte in a solution having a concentration of the analyte within an expected physiological range.

*113.*

118. A method of determining a level of an analyte in an animal, comprising:  
implanting a sensor in the animal, the sensor comprising a substrate, a plurality of conductive traces disposed on the substrate, and a working electrode formed from a one of the plurality of conductive traces,  
generating a signal at the working electrode in response to the analyte;  
analyzing the signal to determine a level of the analyte; and

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producing an electrical current through a portion of the animal, if the level of the analyte exceeds a threshold amount, to warn the animal, the electrical current is produced by applying a potential between two of the conductive traces.

*103*  
119. The method of claim 118, wherein the potential ranges from 1 to 10 volts.

*104*  
120. The method of claim 118, wherein the electrical current ranges from 0.1 to 1 mA.

*105.*  
121. The method of claim 118, wherein the plurality of conductive traces comprises at least three conductive traces and the potential is provided between two conductive traces which do not form the working electrode.

*SUB A121*

122. An electrochemical sensor, comprising:  
a substrate;  
a conductive material disposed on the substrate to form a working electrode; and  
catalyst dispersed in the conductive material, the catalyst catalyzing a reaction of the analyte to generate a signal at the working electrode.

123. The electrochemical sensor of claim 122, wherein the conductive material is carbon.

124. The electrochemical sensor of claim 122, wherein the catalyst is an enzyme.

125. The electrochemical sensor of claim 122, further comprising a binder dispersed in the conductive material.

126. The electrochemical sensor of claim 125, wherein the binder is cured so that the catalyst and conductive material are non-leachably disposed on the substrate.

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